Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested. By the present amendment claim 5 has been amended to recite "human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue". Also, new claim 6 has been added. Support for the present amendment to claim 5 and new claim 6 can be found in U.S. Patent Nos. 5,197,985, and 5,226,914 and PCT Publication No. WO 92/22584, which are incorporated by reference on page 5, lines 2-5 of the present Application. Additionally, Applicants respectfully traverse the anticipation rejection in view of Nolta et al. as evidenced by Prockop and Wells et al. as evidenced by Prockop.

1. 35 U.S.C. §102(b) rejection of claims 3-5

Claims 3-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Nolta et al. (Blood 86:101-110, 1995, Cited Previously) as evidenced by Prockop, D.J. (Science 276:71-74; Cited Previously)

The office action argues that Nolta et al. discloses a transduction method for human CD34 cells in the presence of a stroma generated by human allogeneic bone marrow stromal cells. The Office Action further states that the bone marrow stromal cell population contains isolated mesenchymal stem cells as evidenced by the teachings of Prockop.

Amended claim 5 recites "…co-culturing human hematopoietic progenitor cells with human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue from human mesoderm tissue…"

Nolta et al. teach the transduction of CD34 cells with retroviral vectors in the presence of a stroma generated by human allogeneic bone marrow stromal cells, a human mesoderm tissue. Although bone marrow derived human stroma may include some mesenchymal stem cells, bone marrow human stroma is not equivalent to mesenchymal stem cells which have been isolated, purified and culturally expanded from human mesoderm tissue.

Isolated, purified and culturally expanded mesenchymal stem cells are distinguished from marrow stroma given that MSCs are distinct in morphology and lack surface markers for T and B lymphocytes, macrophages and endothelial cells (see Application pg. 5, lines 14-17). Nolta et al., at best teach the culturing of CD34 cells in the presence of a mesodermic tissue. Nolta et al., do not teach MSCs which have been further isolated, purified and culturally expanded from such a mesodermic tissue.

The mesenchymal stem cells of the present application represent a well characterized isolated cell population which can be prepared in a reproducible manner in contrast to the heterogeneous stromal cell cultures described by Prockop. As discussed in Prockop, these heterogeneous stromal cell cultures contain T and B lymphocytes, macrophages, dendritic cells and endothelial cells.

The examiner argues in response to Applicant's previous argument put forth that the stromal cell population is devoid of most hematopoietic cells and contains MSCs as evidenced by Prockop and that this bone marrow stromal cell population falls within the broad scope mesenchymal stem cells isolated from human mesoderm tissue.

The heterogeneous bone marrow stromal cell population does not fall within the scope of mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue. Once isolated, purified and culturally expanded, the mesenchymal stem cells of the present Application can be distinguished from the more complex cellular environment present in adherent cells of long-term bone marrow stromal cultures.

As discussed above, the MSCs of the present application lack surface markers for T and B lymphocytes, macrophages and endothelial cells. The isolated, purified and culturally expanded mesenchymal stem cells for use in the present invention are prepared using detailed procedures described in U.S. Patent Nos. 5,197,985 and 5,226,914 and PCT Publication No. WO 92/22584 (see Application, pg. 5, lines 2-5). The MSCs for example, can be isolated using a density gradient fractionation, such as by Percoll gradient fractionation or selective antibody purification. The resulting morphologically distinct isolated, purified and culturally expanded mesenchymal stem cell population is not equivalent to the bone marrow stromal cells isolated using the crude plastic adherence methods described in Prockop even if the stromal cells are devoid of most hematopoietic cells and contains some MSCs.

The Examiner also argues that the claims do not require that the MSCs isolated from human mesoderm tissue are homogenous or purified by any particular method, so that no T and B lymphocytes, macrophages and endothelial cells are found. However, amended claim 5 recites human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue. These human

mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue are defined in the specification as a morphologically distinct mesenchymal stem cell population. A cell population that not only is devoid of T and B lymphocytes, macrophages and endothelial cells, but also lacks T and B lymphocytes, macrophages and endothelial cell populations themselves.

The Examiner notes that the instant specification states that "These results demonstrate that hMSCs are able to support ex vivo gene transfer into CD34 human hematopoietic progenitor cells that exhibit transduction efficiencies, cell expansion and drug resistance comparable to the levels produced in Dexter Stroma and FN enhanced transduction, and that Dexter Stroma was derived from adhered bone marrow mononuclear cells that were passaged once.

Applicants fail to see the relevance of the Examiner's statement. The instant specification simply describes experimental results comparing the transduction efficiency of the present method to previously known transduction methods using Dexter Stroma. The statement cited by the Examiner does nothing to equate the cell populations of Dexter Stroma with the MSCs isolated from human mesoderm tissue.

Therefore, the Examiner has failed to show that the heterogeneous adherent bone marrow stromal cells used as feeder layers for hematopoietic stem cells of Prockop are equivalent to the human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue of the present application. Thus, Nolta et al., in view of Prockop et al., does not teach the use of mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue co-cultured with human hematopoietic progenitor cells.

Accordingly, Applicants respectfully request that the 35 U.S.C. §102(b) rejection of claim 5 be withdrawn because Nolta et al. as evidenced by Prockop do not teach all the limitations of claim 5. Claims 3 and 4 depend either directly or indirectly from claim 5, and therefore should be allowed because of the aforementioned deficiencies in the rejection with respect to claim 5 and because of the specific limitation recited in claims 3 and 4.

2. 35 U.S.C. §102(b) rejection of claims 2 and 4-5

Claims 2 and 4-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Wells et al. (Gene therapy 2:512-520, 1995) as evidenced by Prockop, D.J. (Science 276:71-74).

The office action argues that Wells et al. discloses a transduction method for human bone marrow CD34 progenitor cells in the presence of an autologous bone marrow stromal support containing isolated mesenchymal stem cells as evidenced by the teachings of Prockop as described above.

Claim 5 of the present invention is not anticipated by Wells et al. because like Nolta et al., Wells et al. does not teach the co-culturing human hematopoietic progenitor cells with human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue…" At best Wells et al. teach the culturing of CD34 cells in the presence of a mesodermic tissue. Wells et al. do not teach the use of MSCs isolated from such a mesodermic tissue.

As discussed above, the mesenchymal stem cells of the present application represent a well characterized isolated, purified and culturally expanded cell population which can be prepared in a reproducible manner in contrast to the

heterogeneous stromal cell cultures described by Prockop. As discussed in Prockop, these heterogeneous stromal cell cultures contain T and B lymphocytes, macrophages, dendritic cells and endothelial cells. Isolated mesenchymal stem cells of the present invention are distinguished from the complex microenvironment present in marrow stroma given that MSCs are distinct in morphology and lack surface markers for T and B lymphocytes, macrophages and endothelial cells (see Application pg. 5, lines 14-17).

The isolated, purified and culturally expanded mesenchymal stem cells for use in the present invention are isolated and prepared using procedures described in U.S. Patent Nos. 5,197,985 and 5,226,914 and PCT Publication No. WO 92/22584 (see Application, pg. 5, lines 2-5). The MSCs for example, can be isolated using a density gradient fractionation, such as by Percoll gradient fractionation or selective antibody purification. The resulting morphologically distinct isolated, purified and culturally expanded mesenchymal stem cell population is not equivalent to the bone marrow stromal cells isolated using the crude plastic adherence methods described in Prockop.

The examiner argues in response to Applicant's previous argument put forth that the stromal cell population of Wells et al. is depleted of hematopoietic cells and contains MSCs as evidenced by Prockop and that this bone marrow stromal cell population falls within the broad scope mesenchymal stem cells isolated from human mesoderm tissue.

The bone marrow stromal cell population of Wells et al. as evidenced by Prockop does not fall within the broad scope mesenchymal stem cells isolated,

purified and culturally expanded from human mesoderm tissue. As discussed in relation to Nolta et al., bone marrow derived human stroma may naturally include some mesenchymal stem cells. However, bone marrow human stroma depleted of most hematopoietic cells and macrophages is still not an equivalent cell population to mesenchymal stem cells isolated, purified and culturally expanded from mesoderm tissue recited in claim 5.

The Examiner also argues that the claims do not require that the MSCs isolated, purified and culturally expanded from human mesoderm tissue are homogenous or purified by any particular method, so that no T and B lymphocytes, macrophages and endothelial cells are found. However, claim 5 recites human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue. These human mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue as described in the specification are a morphologically distinct mesenchymal stem cell population. The human mesenchymal stem cell population of the present invention is not only described as devoid of T and B lymphocytes, macrophages and endothelial cell populations themselves, but further lacks T and B lymphocytes, macrophages and endothelial cell surface markers.

Furthermore, the Prockop reference states that although the adherent cells used as feeder layers for hematopoietic stem cells have many of the characteristics of mesenchymal stem cells, which are isolated by their adherence to plastic in the absence of non-adherent cells, it is not clear if the adherent cells contain true mesenchymal stem cells. Prockop states that it is uncertain that the adherent feeder

cells retain the potential to differentiate into bone, cartilage, and other mesenchymal cells. Prockop even suggests that the adherent cells may have differentiated into another and discrete phenotype because of their continuing interaction with hematopoietic cells (pg. 72, col. 3).

The Examiner argues that there is no factual evidence indicating that adherent feeder layer cells would not retain the potential to differentiate into bone, cartilage, and other mesenchymal cells. Regardless, the Examiner has still failed to show that adherent feeder layer cells of Wells et al. are equivalent to the mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue the of the present application.

As stated above, the mesenchymal stem cells of the present application represent a well characterized cell population which can be prepared in a reproducible manner in contrast to the heterogeneous stromal cell cultures of Wells et al. The mesenchymal stem cells of the present Application can be distinguished from the more complex cellular environment present in adherent cells of long-term bone marrow stromal cultures.

Again, the Examiner notes that the instant specification states that "These results demonstrate that hMSCs are able to support ex vivo gene transfer into CD34 human hematopoietic progenitor cells that exhibit transduction efficiencies, cell expansion and drug resistance comparable to the levels produced in Dexter Stroma and FN enhanced transduction, and that Dexter Stroma was derived from adhered bone marrow mononuclear cells that were passaged once.

As discussed above, Applicants fail to see the relevance of the Examiner's statement. The instant specification simply describes experimental results comparing the transduction efficiency of the present method to previously known transduction methods using Dexter Stroma. The statement cited by the Examiner does nothing to equate the cell populations of Dexter Stroma with the MSCs isolated, purified and culturally expanded from human mesoderm tissue.

The Office Action fails to show that the heterogeneous adherent bone marrow stromal cells used as feeder layers for hematopoietic stem cells of Wells et al, as evidenced by Prockop are equivalent to the isolated, purified and culturally expanded mesenchymal stem cells of the present application. Therefore, Wells et al, as evidenced by Prockop, has failed to teach the use of mesenchymal stem cells isolated, purified and culturally expanded from human mesoderm tissue co-cultured with human hematopoietic progenitor cells.

Accordingly, Applicants respectfully request that the 35 U.S.C. §102(b) rejection of claim 5 be withdrawn because Wells et al. as evidenced by Prockop do not teach all the limitations of claim 5. Claims 2 and 4 depend either directly from claim 5, and therefore should be allowed because of the aforementioned deficiencies in the rejection with respect to claim 5 and because of the specific limitation recited in claims 2 and 4.

3. New Claim 6

New claim 6 recites the further limitation of "…human mesenchymal stem cells that have been isolated, purified and culturally expanded from a bone marrow specimen by adding the bone marrow specimen to a medium which contains factors

which stimulate mesenchymal cell growth without differentiation…" Support for New claim 6 can be found in U.S. Patent Nos. 5,197,985, and 5,226,914 and PCT Publication No. WO 92/22584, which are incorporated by reference on page 5, lines 2-5 of the present Application.

Claim 6 is patentable because Nolta et al. and Wells et al. as evidenced by Prockop do not teach all the limitations of claim 6.

In view of the foregoing, it is respectfully submitted that the present application is in a condition of allowance and allowance of the present application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this matter to our Deposit Account No. 20-0090.

Respectfully submitted,

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